Analysis of *in vivo* Responses by Mixed-Effect Models

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Objectives. In *in vivo* experimentation, the large intra-group variability between animals is a major obstacle that prevents to **detect significant therapeutic effects** of treatment factors. Our objective is to assess a new statistical method able to better estimate and characterize the additive effects of the combination of an **oncolytic virus** (TG6002) and the **prodrug flucytosine** (5-FC) associated in an anti-cancer treatment. The experimental data are kinetics of tumor growth collected during in vivo assays carried out on mice.

Method

$$y_{ij} = x(t_{ij}, \theta_{\mathbf{i}}) + \epsilon_{\mathbf{ij}}, \quad \forall \mathbf{i} = \mathbf{1}, \ldots, \mathbf{r}, \forall \mathbf{j} = \mathbf{1}, \ldots, \mathbf{n_i},$$

$$x(t_{ij}, \theta_{\mathbf{i}}) = k_{1,i}(t_{ij} + t_0) + x_{0,i} + k_{2,i}(t_{ij} + t_0 - \tau_i)$$

$$\theta_{\mathbf{i}} = \lambda + \mathbf{c_i}\beta + \theta_{\mathbf{i}}, \ \theta_{\mathbf{i}} \sim \mathcal{N}(\mathbf{0}, \mathbf{\Omega}), \quad \forall \mathbf{i} = \mathbf{1}, \ldots, \mathbf{r},$$

$$\theta_{\mathbf{i}} = \begin{pmatrix} x_{0,i} & k_{1,i} & k_{2,i} & \tau_i \end{pmatrix}^{\mathbf{T}}$$

$$\epsilon_{ij} = \sigma \ e_{ij}, \ e_{ij} \overset{i.i.d.}{\sim} \mathcal{N}(0,1), \quad \forall i = 1, \ldots, r, \forall j = 1, \ldots, n_i,$$

$$c_i = \begin{pmatrix} c_{c,i} & c_{d,i} & c_{0,i} \end{pmatrix}$$

$x_{0,i}$	Initial diameter	c_c	TG6002 concentration factor
$k_{1,i}$	First growth rate	c_d	5-FC presence factor
$k_{2,i}$	Second growth rate	c_O	Initial diameter variation
τ_i	Time delay between effects		

The experimental set up is decomposed into 4 main steps:

1. a **full factorial design of experiments** is proposed. TG6002 was tested at four concentrations in combination or not with 5-FC. Eight groups of 13 mice are randomly affected to each experimental condition.
2. we propose a **mixed-effect model** to describe the kinetic growth of the mean tumor diameter.
3. the model parameters are determined with a **maximum likelihood estimator** based on an expectation-maximization algorithm.
4. effects of the two examined components are assessed with a **Wald test.**

Group. No.	TG6002	5-FC
1	0	With
2	0	Without
3	Dose 1	With
4	Dose 1	Without
5	Dose 2	With
6	Dose 2	Without
7	Dose 3	With
8	Dose 3	Without

Tab.1: Full Factorial Design of Experiments with 2 factors

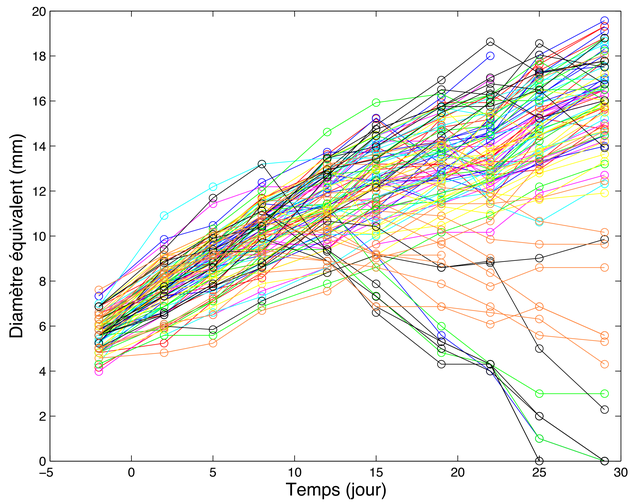

Fig.1: Longitudinal Data: growth kinetics of the tumor equivalent diameter (mm) for 104 mice distributed between 8 groups of treatment: group 1 (magenta), group 2 (red), group 3 (cyan), group 4 (blue), group 5 (yellow), group 6 (green), group 7 (orange), group 8 (black)

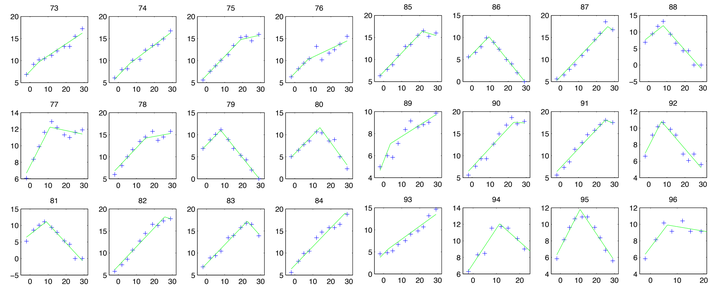

Fig.2: Mixed-effect modeling of the time responses for 24 mice. Measured responses (blue) & Model responses (green)

Results

Our model structure fits correctly all the 104 observed growth responses. The confidence on the estimation results is such that we can detect two tratement effects. Indeed, we show a 3% reduction of the therapeutic response time due to 5-FC and a division by five of the growth delay with TG6002.

Conclusion

Results confirm the relevance of mixed-effect kinetic models to increase the power of statistical tests applied to *in vivo* studies and efficacy of the combination TG6002 with 5-FC.

Effect	Value	P-value
$\beta_{k_2}(D_{TG} = 3)$	-0.42	$1.4\ 10^{-10}$
$\beta_{\tau}(5FC)$	-0.45	$1.7\ 10^{-4}$

Tab.2: Estimated effects of the therapeutic factors on the post-treatment growth rate (k_2) and response delay (τ).Only the 2 most significant effects are indicated here.

Reference

T. Bastogne, A. Samson, P. Vallois, S. Wantz-Mézières, S. Pinel, D. Bechet, and M. Barberi-Heyob. Phenomenological modeling of tumor diameter growth based on a mixed effects model. Journal of Theoretical Biology, 262:544–552, 2010.